26. (clean copy of amended claim) The method of claim 24 wherein the epimerizing enzymes are from the *Pseudomonas putida* FaoAB complex.

Please cancel claims 28-30.

31. (amended) A [genetically engineered organism] transgenic bacterium or plant for use in any of the methods of claims 1-30.

31. (clean copy of amended claim) A transgenic bacterium or plant for use in any of the methods of claims 1-30.

32. (amended) The [organism] transgenic bacterium of claim 31 [wherein the organism is a bacteria].

32. (clean copy of amended claim) The transgenic bacterium of claim 31.

33. (amended) The [organism] transgenic plant of claim 31 wherein the [organism] plant is a higher order plant.

33. (clean copy of amended claim) The transgenic plant of claim 31 wherein the plant is a higher order plant.

Please cancel claim 34.

Remarks

Rejections under 35 U.S.C. §112, first paragraph

Claims 1-27 and 31-33 were rejected under 35 U.S.C. §112, first paragraph, as not enabling. This rejection is respectfully traversed if applied to the amended claims.

The first basis of the rejection is that applicants only provided representative examples of genes that could be used, and did not provide a list of sequences encoding all known enzymes.

However, there is no requirement to do so. Applicants are not claiming the genes *per se* (as was the case with Lilly, where the issue was that applicants only provided a single sequence to support a claim to the entire genus of sequences encoding the same protein but from different organisms). Applicants are instead claiming transgenic organisms where at least one of the genes required for production of PHBH is a transgene which is stably integrated into the chromosome of the organism to make the polymer. The genes are known. All one skilled in the art has to do to obtain numerous sequences encoding these enzymes from a variety of organisms is to look in medline or some other data base, look up the cited example from applicants' specification, and one would obtain other useful sequences. The requirements under 35 U.S.C. §112 are quite clear: one must enable those skilled in the art to make and use that which is claimed, not provide one with everything that is already known.

The second rejection was on the basis that the specification only enables transgenic bacteria encoding a beta-ketoacyl-CoA reductase, beta-ketothiolase, and PHB polymerase that accepts 3-hydroxyhexanoyl CoA and transgenic bacteria encoding a D-specific enoyl-CoA hydratase. The rationale behind this rejection is not apparent, since there is only an allegation (not enabled). However, the burden is on the examiner to do more than simply make the assertion. The examiner must provide some basis for making the rejection. No art has been cited that only one sequence encodes these enzymes, or that the substrate specificity is different among enzymes of the same name. Nor, for that matter, has any reasoning at all been provided for why the claims should be limited to bacteria. Production of polymer in transgenic plants expressing bacterial genes is well known - multiple patents have been issued on the same. The

difference here is the engineering required not just to produce polyhydroxybutyrate, but polyhydroxyhexanoate.

The examples demonstrate reduction to practice in *E. coli*, as the examiner notes. The examiner states that the claims should be limited to a specific source for the enzymes since applicants have only provided a single sequence for each of the polymerase that accepts hydroxyhexanoyl CoA and D-specific enoyl-CoA hydratase. However, as demonstrated in the scientific as well as patent literature, it is well established that one can isolate the genes from other organisms once one has obtained the sequence from the first organism. This is absolutely routine. In the case of the PHB polymerase, the gene from one bacterial species was used to isolate the gene from several species - all of which hybridized under standard conditions to the first gene. This was the basis for issuance of U.S. Patent No. 5,534,432. Genes encoding the enzymes in the PHB and PHA synthetic pathway (beta-ketothiolase, acetoacetyl-CoA reductase and PHB polymerass or PHA polymerase) from *Zoogloea ramigera* strain I-16-M, *Alcaligenes eutrophus*, *Nocardia salmonicolur*, and *P. olevorans* were identified or isolated and expressed in a non-PHB producing organism, *E. coli*. This was based on technology available in 1987. Therefore no undue experimentation is required, once one has the first gene and the function of the encoded protein. In fact, the examiner's attention is drawn to page 13 - note that there are a number of bacterial species which have been reported to be able to utilize substrates within the necessary range (C5-16) to produce PHBH. Note also at the bottom of page 10 that useful synthetases have been isolated from multiple bacterial species, not just one. The claims have been limited to bacterial genes, however, to facilitate prosecution.

With regard to the issue of expressing these bacterial genes in plants, the scientific and patent literature includes numerous examples dating from 1989 to years prior to filing of this application, specifically of the expression of bacterial genes in pathways for the production of polyhydroxyalkanoates in plants. See for example, U.S. Patent No. 6,175,061, 6,091,002, 5,250,430, and 5,610,041. Therefore those skilled in the art would have had no more difficulty in expressing the claimed pathway in plants than they would have in bacteria. The previous reports had demonstrated that bacterial genes encoding enzymes in pathways to make polymer could readily be transferred from bacterial to plants, and produce polymer, in the cytoplasm, in the plastids and in the seeds.

Claims 1, 3, 7-9, 11, 13-27, and 31 have been amended to correct antecedent basis. Claims 6-27 have been rejected as improperly dependent on claim 1. This rejection is inappropriate. The dependent claims further limit the method of claim 1 by limiting the organism, the enzymes, or the conditions under which polymer is made. There is no requirement that the dependent claim must define an additional method step to further limit a method claim. The phrase "derived" has been deleted.

Rejections under 35 U.S.C. §101

The claims have been amended to recite a transgenic bacterium or plant. This moots any concern that the claim might read on a human.

Rejections under 35 U.S.C. §102(a)

Claims 1, 2, 5-11, 15, 31, and 32 were rejected under 35 U.S.C. §102(a) as disclosed by Fukui, et al., J. Bacteriol. 179:4821-4830 (1997). This rejection is respectfully traversed.

The claims require that a transgene encoding either a PHA polymerase incorporating C_6 substrates or D-specific enoyl-CoA hydratase be incorporated into the genome of a plant or bacterium. Fukui does not disclose integration of isolated genes. They demonstrate PHBH production in bacteria by expression of plasmids in the bacteria.

Allowance of all of claims 1-27 and 31-33 as amended is earnestly solicited. All claims as now pending are attached in an appendix to facilitate the examiner's review.

Respectfully submitted,

Patrea L. Pabst
Reg. No. 31,284

Date: February 12, 2001
ARNALL GOLDEN & GREGORY, LLP
2800 One Atlantic Center
1201 West Peachtree Street
Atlanta, Georgia 30309-3450
(404) 873-8594
(404) 873-8595 (fax)

Certificate of Mailing under 37 CFR § 1.8(a)

I hereby certify that this paper, along with any paper referred to as being attached or enclosed, is being deposited with the United States Postal Service on the date shown below with sufficient postage as first-class mail in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

Patrea Pabst

Date: February 12, 2001

APPENDIX: Claims as pending upon entry of amendment

1. (amended) A method for the biological production of [polyhydroxyalkanoates] polyhydroxyalkanoate containing 3-hydroxyhexanoate comprising [synthesizing the polyhydroxyalkanoate in a] growing a transgenic organism selected from the group consisting of a transgenic bacterium and a transgenic plant having at least one bacterial transgene encoding an enzyme selected from the group consisting of [PHB polymerase,] a PHA polymerase incorporating C_6 substrates[, ß-ketothiolase, ß-ketoacyl-CoA reductase,] and a D-specific enoyl-CoA hydratase [, crotonase, butyryl-CoA dehydrogenase, and 3-hydroxybutyryl-CoA dehydrogenase], integrated into the chromosome, under conditions suitable for production of polyhydroxybutyrate-polyhydroxyvalerate by the transgenic organism.
2. (amended) The method of claim 1 wherein the organism is a [bacteria or] plant.
3. (amended) The method of claim 2 wherein the organism is a plant selected from the group consisting of an oil crop [plants] plant and a starch accumulating [plants] plant.
4. (amended) The method of claim 3 wherein the plant is selected from the group consisting of *Brassica*, sunflower, soybean, corn, safflower, flax, palm, coconut, potato, tapioca, cassava, alfalfa, grass, and tobacco.
5. (amended) The method of claim [2] 1 wherein the organism is a [bacteria] bacterium selected from the group consisting of *Escherichia, Klebsiella, Ralstonia, Alcaligenes, Pseudomonas,* and *Azotobacter.*
6. The method of claim 1 wherein the organism is genetically engineered to express or overexpress a PHA polymerase incorporating C_6 substrates.
7. (amended) The method of claim 6 wherein the [enzyme] polymerase is [derived] from *Aeromonas caviae, Comamonas testosteroni, Thiocapsia pfenigii, Chromatium vinosum, Bacillus cereus, Nocardia carolina, Nocardia salmonicolor, Rhodococcus ruber, Rhodcoccus rhodocrous,* and *Rhodospirilum rubrum.*
8. (amended) The method of claim 1 wherein the [organisms are] organism is genetically engineered to redirect metabolites to production of 3-hydroxyhexanoyl-CoA.
9. (amended) The method of claim 8 wherein the [organisms are] organism is genetically engineered using a D-specific enoyl-CoA hydratase gene.
10. (amended) The method of 9 wherein the hydratase gene is isolated from a [bacteria] bacterium selected from the group consisting of *R. eutropha, Klebsiella aerogenes, P. putida,* and *Aeromonas caviae.*
11. (amended) The method of claim 8 wherein the [organisms are] organism is genetically engineered using the genes encoding the enzymes in a butyrate fermentation pathway.
12. (amended) The method of claim 11 wherein the enzymes in the butyrate fermentation pathway [is] are from *Clostridium acetobutylicium* or *Thermoanaerobacterium thermosaccharolyticum.*
13. (amended) The method of claim 11 wherein the [organisms are] organism is genetically engineered to convert butyrate to butyryl CoA or butyryl CoA to crotonyl CoA.
14. (amended) The method of claim 11 wherein the [organisms are] organism is genetically engineered to express a broad range reductase that is active on C_6 substrates.

15. (amended) The method of claim 11 wherein the [organisms are] organism is genetically engineered to express a polymerase that accepts 3-hydroxyhexanoyl CoA.
16. (amended) The method of claim 11 wherein the [organisms are] organism is genetically engineered to express a thiolase accepting acetoacetyl CoA.
17. (amended) The method of claim 11 wherein the [organisms are] organism is genetically engineered to express an enzyme selected from the group consisting of thiolases specific for 3-ketohexanoyl CoA, reductase active on 3-ketohexanoyl CoA, [PHA polymerase that accepts 3-hydroxybutyryl CoA] and 3-hydroxyhexanoyl CoA.
18. (amended) The method of claim 8 wherein the [organisms are] organism is further genetically engineered [using] to express one or more fatty acid biosynthetic enzymes.
19. The method of claim 18 wherein the fatty acid biosynthetic enzymes are enzymes converting acyl ACP to acyl CoA.
20. The method of claim 19 where the enzymes are selected from the group consisting of ACP-CoA transacylase, acyl ACP thioesterase, and acyl CoA synthase.
21. The method of claim 20 wherein the enzymes are acyl ACP thioesterase and acyl CoA synthase.
22. (amended) The method of claim 18 wherein the enzymes are [derived] from *E. coli*.
23. (amended) The method of claim 8 wherein the [organisms are] organism is further genetically engineered [using a] to express one or more enzymes forming a fatty acid oxidation complex.
24. The method of claim 23 wherein the fatty acid oxidation complex comprises enzymes selected from the group consisting of enzymes epimerizing S-3 hydroxyhexanoyl CoA and enzymes reducing 3-ketohexanoyl CoA.
25. (amended) The method of claim 24 wherein the enzymes are [derived] from *Nocardia salmonicolor*.
26. (amended) The method of claim 24 wherein the epimerizing enzymes [for epimerization] are [derived] from the *Pseudomonas putida* FaoAB complex.
27. The method of claim 23 wherein the organism that is genetically engineered accumulates 3-ketohexanoyl CoA due to a lack of a thiolase.
31. (amended) A [genetically engineered organism] transgenic bacterium or plant for use in any of the methods of claims 1-30.
32. (amended) The [organism] transgenic bacterium of claim 31 [wherein the organism is a bacteria].
33. (amended) The [organism] transgenic plant of claim 31 wherein the [organism] plant is a higher order plant.
Please cancel claim 34.